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IN VITRO GLUCOSE METABOLISM IN THE EPIDERMIS AND DERMIS OF THE PIG

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Glucose metabolism was studied in skin slices prepared with a dermatome and obtained from the mid-lumbar region of young pigs. The first slice, comprising the epidermis was 0.1 mm thick, whereas all subsequent slices were prepared from the dermis and were 0.2 mm thick. Each precooled slice was incubated for 2 hours at 37°C in a Dubnoff metabolic incubator in 3 ml of Krebs-Ringer buffer containing 30 µmoles glucose and 0.5 µCi of U-C^{14}-glucose. At the end of the incubation period glucose oxidation and glucose incorporation into various metabolites were determined. The

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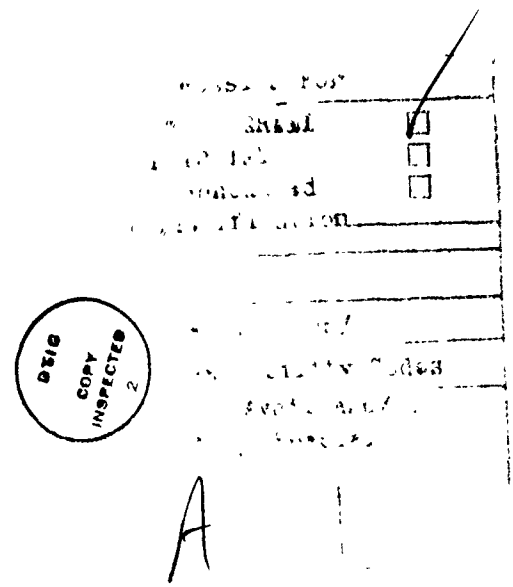
results show that glucose oxidation was most active in the epidermis and the first section of the dermis. In the deeper layers of the dermis glucose oxidation was markedly dimished and was minimal in the central region of the dermis. Thereafter, glucose oxidation gradually increased and approached a level similar to that observed in the upper layer of the dermis. Fatty acids and glycerol synthesis followed a similar pattern. In contrast, glucose incorporation into glycogen and the non-saponifiable fraction was uniformly distributed throughout the various layers of the skin. Exogenous insulin enhanced glucose oxidation, fatty acids and glycogen synthesis but had no effect on the synthesis of glycerol and non-saponifiable lipids. Glucagon did not affect any of the processes studied. The findings in this study demonstrate that the skin, and in particular, the dermis is not a metabolically homogeneous tissue.

ABSTRACT

Glucose metabolism was studied in skin slices prepared with a dermatome and obtained from the mid-lumbar region of young pigs. Eight slices, equivalent to 1.5 mm skin thickness were prepared. The first slice (0.1 mm thick) was primarily epidermis, all subsequent slices (0.2 mm thick) were dermis. Each precooled slice was incubated for 2 hours at 37 C in a Dubnoff metabolic incubator in 3 ml of Krebs-Ringer buffer containing 30 μmoles glucose and 0.5 μCi of C^{14}-U-glucose. At the end of the incubation period glucose oxidation and glucose incorporation into various metabolites were determined. The results show that glucose oxidation was most active in the epidermis and the first section of the dermis. In the deeper layers of the dermis glucose oxidation was markedly diminished and was minimal in the central region of the dermis. Thereafter, glucose oxidation gradually increased and approached a level similar to that observed in the upper layer of the dermis. Fatty acids and glycerol synthesis followed a similar pattern. In contrast, glucose incorporation into glycogen and the non-saponifiable fraction was uniformly distributed throughout the various layers of the skin. Exogenous insulin enhanced glucose oxidation, fatty acids and glycogen synthesis but had no effect on the synthesis of glycerol and non-saponifiable lipids. Glucagon did not affect any of the processes. The findings in this study demonstrate that the skin, particularly the dermis, is not a metabolically homogeneous tissue.



PREFACE

An affiliation program between LAIR and the University of San Francisco was established in 1980. Under this program qualified students from USF actively participate in research conducted at LAIR and their efforts are credited toward the course work at the University. Some of the data presented in this report were used by Jorge Palafox in partial fulfillment of the requirment for graduate research in Biology 220 at USF.

We wish to express our appreciation to CPT Warren W. Jederberg, MS, for the preparation of tissue slices used in these studies.

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Skin, one of the largest organs of the human body, is frequently overlooked when assessing the importance of individual organs or tissues for the maintenance of homeostasis. Without the skin as a protective envelope, the body would rapidly equilibrate with its environment — a situation not compatible with life. The conventional view of the skin has stressed its function as a passive barrier. However, the skin also has the capacity to metabolize endogenous substrates, as well as drugs and other exogenous chemicals. Some of these activities such as the formation of sebum and the process of keratinization are unique to the specialized functions of the skin. They take place simultaneously with biochemical activities which are related to cellular proliferation and general metabolism, such as DNA synthesis and glycolysis.

In this paper, a brief initial review of cutaneous anatomy and physiology will summarize current understanding of selected structural and functional characteristics of the skin. This is followed by an outline of metabolic activity in the skin to emphasize that the skin is far from an inert tissue functioning simply as a passive barrier but rather should be thought of as a tissue capable of active and diverse patterns of cellular metabolism.

Skin Structure and Function: Mammalian skin consists of two distinct tissue components: the thinner, outer, stratified squamous epithelium known as the epidermis and the thicker underlying dermis. Each has unique features. In addition to these two components, there are several other structures including eccrine and apocrine sweat glands, sebaceous glands and hair follicles present in the skin. Each of these structures are located primarily in the dermis.

Epidermis: The epidermis consists of a basal layer of germinating keratinocytes in direct and tight apposition to the underlying dermis, and a variable number of differentiating squamous cells. The phenomenon of epidermal differentiation, is normally an orderly process whereby keratinocytes migrate upward from the basal layer of the epidermis and in the process produce a tough, fibrous protein known as keratin. Keratin is an α-helical protein with a molecular weight of approximately 60,000 - 68,000. It is relatively poor in sulfur-containing amino acids but is rich in glycine, serine, glutamic and aspartic acids (1). A second, basic protein has also been isolated and purified from rat epidermis (2). It has a

molecular weight of about 40,000 and its amino acid composition differs substantially from that of the α-helical fibrous protein. Other cells present in the epidermis include melanocytes which produce melanin, a pigment that functions to protect the skin against deleterious effects of UV radiation. A third epidermal cell, known as the Langerhans cell appears to share certain characteristics with macrophages and may play an important role in the processing of contact allergens by the skin (3). The epidermis does not have blood vessels, therefore injury to the epidermis alone does not cause bleeding.

Dermis: The thicker component of the skin, upon which the epidermis rests and to which it is tightly bound, contains abundant structural proteins, such as collagen and elastin embedded in a ground substance of glycosaminoglycans. The cells of the dermis include fibroblasts which synthesize and secrete these structural proteins, fixed macrophages, eosinophils, basophils, neutrophils and mast cells. The dermis is rich in blood vessels and nerves, both of which are absent in the epidermis. The dermis with its rich vascularity has an important thermoregulatory function. Sebaceous glands actively synthesize a complex series of lipids, the exact role of which remains controversial (4). Hair follicles are among the most rapidly replicating of mammalian tissues, and extensive studies have verified the usefulness of studying metabolic activity of these skin organelles to diagnose a number of gene effects in man (5).

Skin Metabolism: A perusal of the literature indicates that the skin is capable of a wide variety of biochemical activities. The skin of an average man has been estimated to weigh about 4 kg (or about three times as much as his liver) (6). The sheer size of the pool of skin metabolites is therefore a factor that deserves attention in examining the overall metabolism of man.

Carbohydrate Metabolism: Enzymes integral to aerobic and anaerobic glycolysis are present in cutaneous tissue (7-9). Most of the enzymes of carbohydrate metabolism found in other tissues also have been identified in skin including hexokinase, phosphoglucomutase and glycogen synthetase. Skin glucose levels are approximately 40 - 50% of blood glucose levels. The use of pathways of glucose metabolism in skin differs considerably from that of other tissues, such as liver, in that anaerobic glycolysis predominates and lactate is a major end-product of cutaneous glucose metabolism. Less than 2% of glucose in human skin enters the tricarboxylic acid cycle whereas more than 70% is converted to lactate (10). The hexosemonophosphate shunt is also highly active in skin. Because of the relative inefficiency of skin's predominant carbohydrate metabolic pathway in generating ATP, a major source of energy in cutaneous tissue is derived from fatty acid oxidation (10).

Lipid Metabolism: Skin surface lipids are derived from two major sources, the epidermis and sebaceous glands. Sebum, the secretory

product of sebaceous glands, is a major component of human skin surface lipids. Several different categories of lipids are present in human skin, including triglycerides, waxes and waxy esters, free fatty acids, squalene and sterols (4). Human skin is active in lipogenesis and fatty acids up to a chain length of C-30 are produced in the skin (4). Lipid synthesis occurs in both the epidermis and dermis (11).

Protein Metabolism: The skin is one of the most active body tissues insofar as protein synthesis is concerned (12). Extensive studies have confirmed that isotopically labelled amino acids are incorporated into epidermal cells at all levels. Specific amino acids are incorporated in specific epidermal regions. Thus, glycine, leucine, and alanine are incorporated primarily in the basal-cell layer, whereas arginine, histidine and methionine are incorported primarily in the granular cell layer of neonatal rat epidermis (13). These differential patterns of amino acid uptake no doubt reflect the nature of the proteins synthesized in the skin layers.

Control of Metabolism: Limited data indicate that hormones and metabolic intermediates participate in the control of skin metabolism. Thus, the skin of alloxan induced diabetic rats has impaired lipogenic activity which can be corrected with insulin treatment (14). Starvation also decreases lipid synthesis in the skin and refeeding reverses this process. The level of glycerol-3-phosphate in the skin fluctuates in parallel with the lipogenic activity, suggesting a possible regulatory role for this intermediate (15). Testosterone and progesterone enhance the reactions between mevalonate and squalene in the pathway of cholesterol synthesis.

Distribution of Metabolic Processes in the Epidermis and Dermis: Numerous experimental data in the literature concerned with metabolic activity in the skin were obtained from whole skin preparations. This experimental approach does not assess the relative contribution of the epidermis and the dermis to metabolic activity of the skin. Functional characteristics of the two skin components are different and consequently, the rates and the distribution of specific metabolic processes may also differ. Indeed, it has been shown that the synthesis of polar lipids from acetate is more active in the epidermis than in the dermis (11). Presently, no information is available as to whether or not metabolic processes in the dermis are uniformly distributed throughout the tissue. As the dermis is a site of vigorous metabolic activity, including the synthesis and degradation of structural and soluble proteins, it is possible that some regions of the dermis are more active than others. This possibility, however, remains to be verified. In this respect, pig skin seems to be an appropriate experimental model. As pig dermis is quite thick, it can be horizontally sectioned and each section subjected to analyses. Glucose was an appropriate substrate in such studies, for glucose carbons enter most of the metabolic pools. In

view of these considerations, in vitro glucose utilization was studied in the epidermis and in various layers of dermis obtained from the domestic pig. In addition, the effects of insulin and glucagon on these processes were investigated, as the two hormones regulate the activity of numerous metabolic pathways.

EXPERIMENTAL DESIGN

Five young female Yorkshire pigs weighing approximately 50 kg were used. The pigs were sacrificed and skin slices were obtained with a dermatome from the clipped mid-lumbar region. Eight slices equivalent to 1.5 mm thickness, were prepared. Histological examination indicated that the first slice (0.1 mm thick) was primarily epidermis, all subsequent slices (0.2 mm thick) were dermis. The slices were cooled in an ice-cold solution of saline, weighed and incubated in 25 ml Erlenmeyer flasks containing 3.0 ml of Krebs-Ringer bicarbonate buffer, 30 μ moles of glucose and 0.25 μCi of C^{14}-U-glucose. Where applicable 0.1 unit of porcine insulin or 0.1 mg of crystalline glucagon was added to the incubation medium. The flasks were gassed for 1 minute with 95% O_2-5% CO_2 and stoppered with a rubber stopper from which a plastic well was suspended. The flasks were incubated in a Dubnoff shaker for 2 hrs. at 37 C at 100 strokes/minute. At the end of the incubation period, glucose oxidation and glucose incorporation were measured according to the methods described previously (16). Glucose incorporation into tissue metabolites was expressed as micromoles per gram of tissue in 2 hours. The data were evaluated by the analysis of variance. Differences at $P<0.05$ were considered significant. Differences between means were further evaluated by the Newman-Keuls test (17).

RESULTS

As indicated in Table 1, glucose oxidation in the epidermis was more active than that observed in the dermis. Glucose oxidation in the dermis was most active in the upper most layer of the tissue and least active in the central region of the dermis. Thereafter, the oxidation gradually increased and approached a level similar to that observed in the top region of the dermis. Insulin enhanced glucose oxidation in the epidermis by approximately 40% and by about 59% in the dermis. Glucagon had no effect on glucose oxidation.

The data showing the synthesis of fatty acids in the epidermis and the dermis are given in Table 2. The most active fatty acid synthesis was observed in the epidermis. The most active synthesis in the dermis occurred in the upper regions and the least activity was observed in the central portions of the tissue. However, the synthesis in the deeper regions of the dermis approached the values observed in the upper regions of the tissue. Insulin doubled the synthesis both in the epidermis and the dermis. Fatty acids synthesis was not affected by glucagon.

As shown in Table 3, glycogen synthesis was uniformly distributed throughout the dermis and the activity was equal to that found in the epidermis. Insulin stimulated glycogen synthesis in both tissues but glucagon had no effect. The data showing glycerol synthesis are summarized in Table 4. The synthesis in the epidermis was about 44% higher than in the upper region of the dermis. Again, the activity in the dermis was not uniformly distributed. The activity in the upper and the lower regions of the dermis was twice as high as the activity in the central region. The activity was not affected by the hormones. As indicated in Table 5, the synthesis of non-saponifiable lipids was uniformly distributed throughout the dermis and the activity was similar to that found in the epidermis. The hormones had no effect on the synthesis of non-saponifiable lipids.

DISCUSSION

The results obtained in this study confirm observations of many others (1) who found skin is an active site of numerous and diverse metabolic transformations. Our findings demonstrate that the metabolic activity is more pronounced in the epidermis than in the dermis. Our findings also demonstrate that glucose oxidation and fatty acid and glycerol synthesis are more active in the upper and the lower regions of the dermis than in the central region of the tissue, while glycogen, cholesterol and sterol syntheses are uniformly distributed throughout the dermis. Thus, in many respects the dermis can be regarded as a metabolically heterogeneous tissue. At the present time there is no obvious explanation as to why a particular metabolic sequence is less active in the central than in the peripheral regions of the dermis. The distribution of structural proteins such as collagen and elastin and of soluble proteins in the tissue undoubtely affects metabolic activity in the dermis. As numerous enzymes associated with the disposition of glucose carbons are soluble proteins, a higher metabolic activity would be expected in the regions in which such proteins predominate. The outermost layer of the dermis, the papillary part, consists of a relatively cellular, loose connective tissue with collagen and elastic fibers smaller in diameter and fewer in number than are encountered in the remaining reticular dermis. In the reticular portion of the dermis, the meshes of collagen and elastic fibers are more dense, the fibers are thicker relatively few cells and less ground substance are present (18). In this portion of the dermis a lower metabolic activity would be anticipated. Hair follicles are other components which could contribute to the over-all metabolic activity in the deeper regions of the dermis. Extensive glucose and lipid metabolism and the presence of appropriate enzymes in hair follicles have been demonstrated (19). The assessment of other factors which control metabolic activity in various portions of the dermis is rather difficult due to the complex nature of the tissue. In the present study, glucose oxidation, fatty acid and glycogen synthesis responded to exogenous insulin and not to glucagon. Thus, the distribution of hormone receptors on the membrane or in the cytoplasm of the cells of

the dermis could, at least in part, account for the metabolic heterogeneity observed in this tissue. Glucagon receptors are apparently absent in the skin, since none of the processes studied responded to the hormone. This is in a direct contrast to glucagon effects observed in other tissues (20). Insulin and glucagon have antagonistic biological actions, mediated by protein kinases and protein phosphorylation and dephosphorylation. As glucagon had no effect on glucose oxidation, lipids or glycogen synthesis, the mechanisms of insulin effects in the skin may be different from those in other tissues or organs. Vigorous oxidation of glucose in the epidermis and the dermis suggests the presence of the tricarboxylic acid cycle, the pentose shunt and the mechanism for fatty acid oxidation. Observations in the literature suggest that the pentose shunt predominates and the tricarboxylic acid cycle acitivity is relatively minor (10). A high activity of fatty acid synthesis in the epidermis supports this conclusion. The pentose phosphate shunt and malic enzyme probably supply all the NADPH required for active synthesis of lipids in the skin. The contribution of each source to the NADPH pool in the epidermis and the dermis remains to be determined. Our data, showing that the skin is an active site of sterol metabolism, confirm similar observations of many others (21).

CONCLUSIONS

The study demonstrates that the epidermis and the dermis utilize many of the metabolic pathways found in other body tissues, and that such pathways are more active in the epidermis than in the dermis. The study further demonstrates that the dermis is not a metabolically homogeneous tissue. Some metabolic events are more active in the peripheral regions of the dermis than in the central regions of the tissue. In contrast, other metabolic events may be uniformly distributed throughout the dermis. The significance of these metabolic arrangements is not readily apparent. However, they may be related to the unique structure of the skin and to its function as an environmental interface.

RECOMMENDATIONS

. The critical biochemical and physiological factors controlling metabolic events in the skin should be delineated.

. Human skin should be tested by the same method as we have studied pig skin to determine if our results are applicable.

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APPENDIX

TABLE 1

GLUCOSE OXIDATION BY THE EPIDERMIS AND THE DERMIS

Tissue	Slice	Skin Depth (mm)	Controls	Insulin	Glucagon
Epidermis	1	0-0.1	13.13±0.71[1]	18.41±0.64*	12.91±1.13
Dermis	2	0.1-0.3	8.61±0.53	13.72±0.58*	8.34±0.94
	3	0.3-0.5	6.34±0.59	8.82±0.72*	4.67±0.54
	4	0.5-0.7	2.95±0.46	4.28±0.49*	2.76±0.39
	5	0.7-0.9	3.64±0.73	7.52±0.36*	3.86±0.46
	6	0.9-1.1	5.95±0.46	8.42±0.36*	5.22±0.68
	7	1.1-1.3	7.69±0.64	12.18±0.59*	7.53±0.74
	8	1.3-1.5	8.40±0.78	12.51±0.48*	9.53±0.88

*Indicates significant difference from controls, $P<0.05$, same slice.

[1]Values are μmoles glucose oxidized/g tissue/2 h, mean ± SE from five pigs.

TABLE 2

FATTY ACIDS SYNTHESIS BY THE EPIDERMIS AND THE DERMIS

Tissue	Slice	Skin Depth (mm)	Controls	Insulin	Glucagon
Epidermis	1	0-0.1	1.85±0.18[1]	2.98±0.12*	1.65±0.09
Dermis	2	0.1-0.3	0.92±0.12	1.74±0.09*	1.09±0.09
	3	0.3-0.5	0.28±0.04	0.59±0.05*	0.32±0.10
	4	0.5-0.7	0.11±0.03	0.29±0.07*	0.14±0.04
	5	0.7-0.9	0.19±0.04	0.36±0.09*	0.10±0.08
	6	0.9-1.1	0.76±0.12	1.47±0.13*	0.69±0.07
	7	1.1-1.3	0.64±0.05	1.39±0.12*	0.65±0.07
	8	1.3-1.5	0.75±0.10	1.40±0.08*	0.70±0.12

*Indicates significant difference from controls, $P<0.05$, same slice.

[1]Values are μmoles glucose incorporated/g tissue/2 h, mean ± SE from five pigs.

TABLE 3

GLYCOGEN SYNTHESIS BY THE EPIDERMIS AND THE DERMIS

Tissue	Slice	Skin Depth (mm)	Controls	Insulin	Glugacon
Epidermis	1	0-0.1	0.43±0.09[1]	0.82±0.07*	0.44±0.12
Dermis	2	0.1-0.3	0.53±0.11	0.89±0.10*	0.65±0.10
	3	0.3-0.5	0.38±0.08	0.74±0.12*	0.56±0.18
	4	0.5-0.7	0.49±0.05	0.83±0.13*	0.33±0.10
	5	0.7-0.9	0.47±0.07	0.79±0.08*	0.59±0.09
	6	0.9-1.1	0.39±0.10	0.76±0.09*	0.48±0.12
	7	1.1-1.3	0.52±0.08	0.86±0.10*	0.49±0.13
	8	1.3-1.5	0.40±0.10	0.74±0.08*	0.41±0.08

*Indicates significant difference from controls, $P<0.05$, same slice

[1]Values are μmoles glucose incorporated/g tissue/2 h, mean ± SE from five pigs.

TABLE 4

GLYCEROL SYNTHESIS BY THE EPIDERMIS AND THE DERMIS

Tissue	Slice	Skin Depth (mm)	Controls	Insulin	Glucagon
Epidermis	1	0-0.1	44.20±3.81[1]	45.17±3.71	40.24±4.10
Dermis	2	0.1-0.3	30.76±2.65	28.93±2.01	30.18±3.60
	3	0.3-0.5	24.10±3.12	26.45±2.67	25.83±3.01
	4	0.5-0.7	15.81±1.94	18.72±2.10	16.35±2.48
	5	0.7-0.9	20.46±2.10	19.57±2.70	19.73±2.10
	6	0.9-1.1	27.61±2.76	25.90±1.40	26.40±2.56
	7	1.1-1.3	26.49±1.98	27.18±1.95	25.93±2.41
	8	1.3-1.5	30.12±2.46	28.37±2.91	29.36±3.18

[1]Values are nanomoles glucose incorporated/g tissue/2 h, mean ± SE from five pigs.

TABLE 5

NON-SAPONIFIABLE LIPIDS SYNTHESIS BY THE EPIDERMIS AND THE DERMIS

Tissue	Slice	Skin Depth (mm)	Controls	Insulin	Glucagon
Epidermis	1	0-0.1	1.21±0.18[1]	1.42±0.18	1.11±0.08
Dermis	2	0.1-0.3	1.37±0.14	1.03±0.11	1.23±0.14
	3	0.3-0.5	0.95±0.21	1.29±0.14	1.07±0.15
	4	0.5-0.7	1.34±0.15	1.16±0.12	1.22±0.09
	5	0.7-0.9	1.10±0.09	1.37±0.19	1.24±0.19
	6	0.9-1.1	1.03±0.10	0.93±0.23	0.99±0.05
	7	1.1-1.3	1.23±0.14	1.03±0.10	1.12±0.12
	8	1.3-1.5	1.12±0.12	1.31±0.13	1.21±0.09

[1]Values are μmoles glucose incorporated/g tissue/2 h, mean ± SE from five pigs.

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